Rapid Analysis of Herbicides by Rapid Resolution LC with Online Trace Enrichment

Application

Environmental

Author

Michael Woodman
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

Environmental and Food Safety agencies are constantly updating methods to improve detection limits and to resolve interfering compounds. One particular method, EPA 555, is used for the analysis of chlorinated phenoxy acid herbicides in drinking water. A mandated trace enrichment step significantly impacts the ease of use and reliability of the method. The method uses 5-µm analysis columns and online trace enrichment. The variation here uses small ZORBAX 3.5- and 1.8-µm RRHT columns and an autoSPE (Solid Phase Extraction) cartridge with an automated switching valve mounted in the column compartment. Combined with sample introduction via direct injection to the autoSPE cartridge, instead of the loading pump specified in the EPA method, we dramatically reduce the overall analysis time and virtually eliminate the potential of sample cross-contamination.

Introduction

Trace analyte detection in relatively clean matrices is an excellent application for online SPE procedures. Compared to manually loading samples with disposable SPE cartridges, which require an elution step into a vial prior to analysis, online SPE assures 100% sample transfer to the analysis column and dramatically increases sensitivity by increasing the analyte mass delivered to the column. In EPA Method 555, 20 mL of drinking water is loaded through a pump to an SPE cartridge mounted on a high-pressure switching valve on the HPLC system. Because few, if any, autosamplers can inject this large volume, the sample must be pumped onto the cartridge. Contamination of the loading pump with prior samples is always a concern, and adequate flushing and blank runs become an important part of the overall method procedure.

To reduce the sampling volume sufficient for available automatic preparative samplers, without losing sensitivity in the method, it is necessary to reduce the analysis column size while preserving resolving power. Ancillary benefits of using smaller columns generally include reduced analysis time and solvent consumption, and greater compatibility with ionization sources in mass spectrometers.

If the ratios of their column length to particle size are equal, columns are considered to have equal resolving power. Significant reductions in column volume can be made by reducing the length and/or internal diameter of the column. In the latter case, the flow rate would normally be reduced as in Equation 1.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam.}_{\text{column2}}}{\text{Diam.}_{\text{column1}}}\right]^2 = \text{Flow}_{\text{col. 2}} \quad \text{(eq. 1)}$$

The combined effect of reduced length and diameter contributes to a reduction in solvent consumption. We normally scale the injection mass to the size of the column and a proportional injection volume would be calculated from the ratio of the void volumes of the two columns, multiplied by the injection volume on the original column, as in Equation 2 below.

$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj. vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

Short columns packed with small particle sizes are typically operated at high linear velocities. The increase in elution speed will decrease absolute peak width and may require the user to adjust data acquisition rates and reduce signal filtering parameters. This will ensure that the chromatographic separation is accurately recorded in the acquisition data file.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. We can express the gradient slope as in Equation 3.

$$\%\ \text{Gradient slope} = \left[\frac{(\text{End\%} - \text{Start\%})}{\#\text{Column volumes}}\right] \quad \text{(eq. 3)}$$

Note that the use of % change per column volume rather than % change per minute frees the user to control gradient slope by altering gradient time and/or gradient flow rate. A large value for gradient slope yields very fast gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Longer analysis time may also result unless the gradient slope is reduced by increasing the flow rate, within acceptable operating pressure ranges, rather than by increasing the gradient time.

Resolution increases with shallow gradients because the effective capacity factor, k*, is increased. Much like in isocratic separations, where the capacity term is called k', a higher value directly increases resolution. The effect is quite dramatic up to a k value of about 5–10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

Experimental Conditions

See figure 1 for configuration.

System

Agilent 1200 Series Rapid Resolution LC consisting of:
G1379B micro degasser
G1312B binary pump SL
G1312A binary pump with solvent selection valve option, or
G1354A quaternary pump
G1367C HiP ALS autosampler SL, and
G2258A Dual Loop Prep autosampler 5 ml
G1316B Thermostatted column compartment SL with 6- or 10-port 2-position switching valve
G1315C UV/VIS diode array detector (DAD) SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01

Columns

Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm
Agilent ZORBAX SB-C18, 2.1 × 80 mm, 1.8 µm
Agilent ZORBAX SB-Aq, 4.6 × 12.5 mm, 5 µm

Mobile phase conditions

Organic solvent:	Acetonitrile
Aqueous solvent:	25 mm phosphoric acid in Milli-Q water

Gradient conditions

Gradient slope:	7.8 or 2.3% per column volume, as indicated. See individual chromatograms for flow rate and time

Sample

EPA 555 Group A chlorinated phenoxy acid herbicides (picloram, chloramben, dicamba, bentazon, 2,4-D, dichlorprop, 2,4,5-TP, acifluorfen), 100 µg/mL in methanol or diluted to 20 ng/L (20 ppb) in reagent water acidified with 25 mm phosphoric acid.

Results

The separation was initially performed via direct injection of concentrated standard on a 4.6 × 250 mm, 5-µm ZORBAX SB-C18 column, thermostatted to 25 °C, using conditions referenced in US EPA method 555 (Figure 2). The described trace enrichment procedure using pump A as the loading pump was performed (Figure 3). The method was then scaled in flow and time for exact translation to a 3.0 × 150 mm 3.5-µm column (Figure 4) using 5-mL trace enrichment injection. Finally, a 2.1 × 80 mm 1.8-µm configuration (50-mm plus 30-mm columns in series) is used to demonstrate the feasibility of this separation under conditions for trace enrichment requiring less than 1.5-mL injection. (Figure 5)

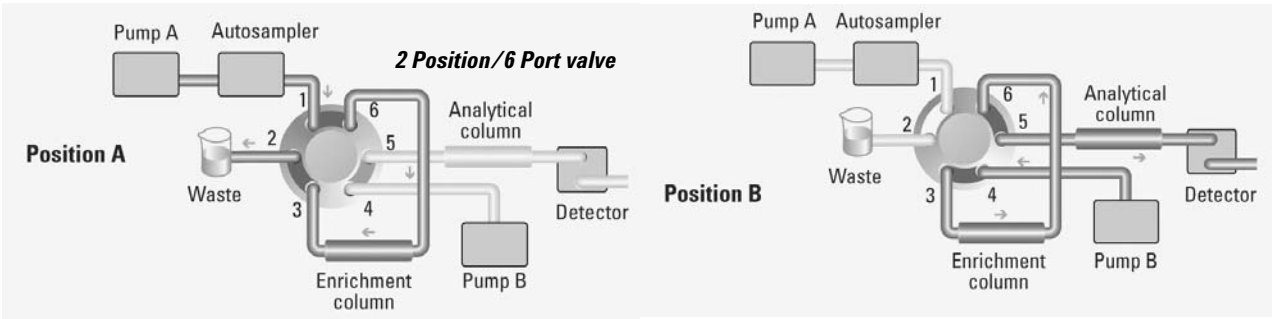


Figure 1. Trace enrichment autoSPE scheme.

Figure 1 shows the schematic placement of modules and columns in the system. The A pump is the loading pump in case of volumes exceeding the 5-mL capacity of the G2258A Dual Loop Autosampler, thus pump A uses one line for sample and a second line for the aqueous eluent, 25 mm phosphoric acid. If direct injection from the autosampler is used, pump A is delivering 25 mm phosphoric acid. If the A pump is fitted with a degasser, the sampling line should bypass the degasser module to minimize contamination with sample solutions. To conduct sampling through the A pump, the valve should be in position B while the new sample is flushed through the A pump. Then switch the valve to the A position and load the required 20 mL sample volume. The analysis begins when the valve is returned to the B position, at which time the sampling line on the A pump would be flushed with reagent water or the next sample, as appropriate.

Figures 2 and 3 show the standard separation by direct injection and pumped trace enrichment, respectively. With column regeneration steps, this results in a total analysis time of 60 minutes. Translation of the gradient to the 3.0- × 150-mm column requires a reduction in flow rate, due to the smaller diameter, and a reduction in gradient time because of the shorter column length. The resulting analysis is reduced from 60 to 36 minutes and solvent consumption is proportionately reduced from 60 mL to 15.5 mL.

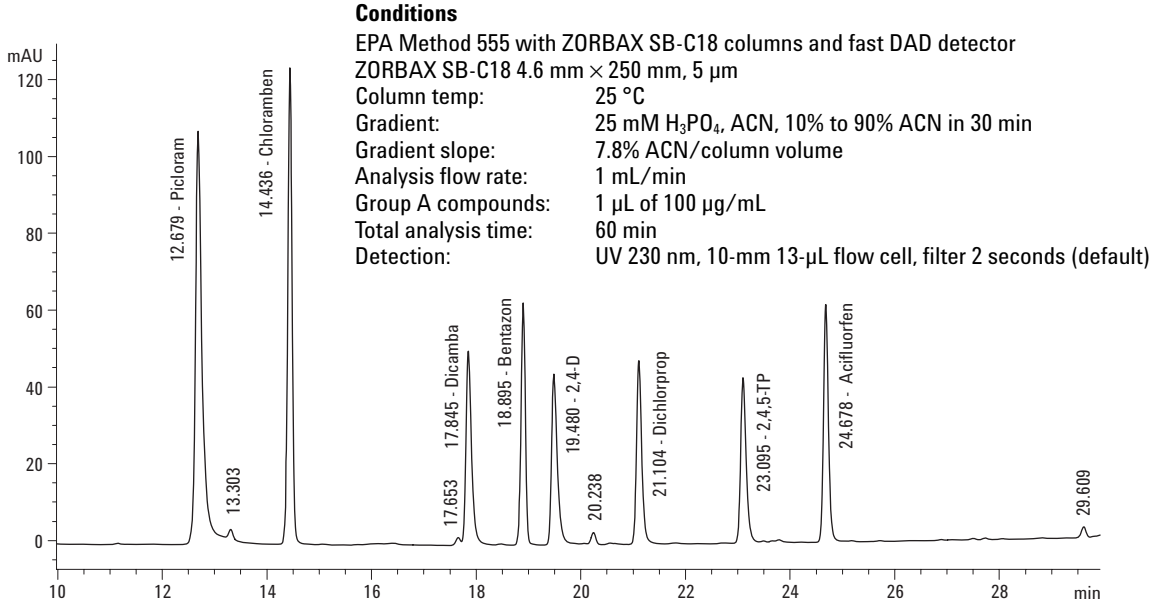
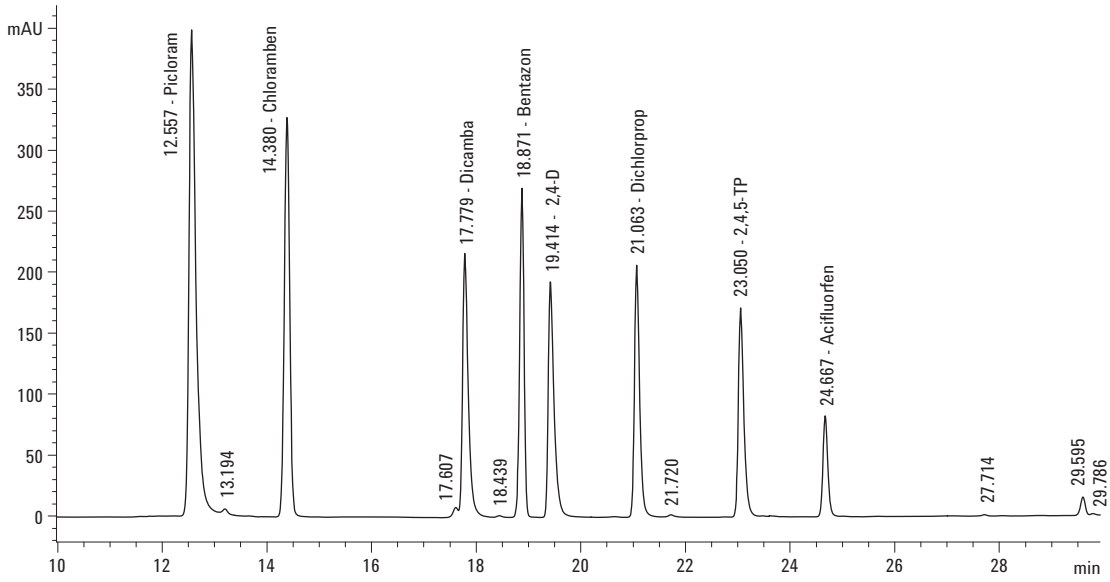


Figure 2. Gradient separation of herbicides on 4.6 mm × 250 mm, 5 µm ZORBAX SB-C18.



Conditions

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 4.6 mm × 250 mm, 5 µm	
Column temp:	25 °C
Gradient:	25-mM H_3PO_4, ACN, 10% to 90% ACN in 30 min
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	1 mL/min
Group A compounds:	20 mL of 20 µg/L trace enrichment
Total analysis time:	60 min
Detection:	UV 230 nm, 10-mm 13-µL flow cell, filter 2 seconds (default)

Figure 3. Trace enrichment (20 mL) of 20-ppb solution on 4.6 × 250 mm 5-µm ZORBAX SB-C18.

Conditions:

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 3 mm × 150 mm, 3.5 µm	
Column temp:	25 °C
Gradient:	25 mm H_3PO_4, ACN, 10% to 90% ACN in 18 min
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	0.43 mL/min
Group A compounds:	5 mL of 20 µg/L (20 ppb)
Detection:	UV 230 nm, 3-mm 2-µL flow cell, filter 0.2 seconds
Total analysis time:	36 min

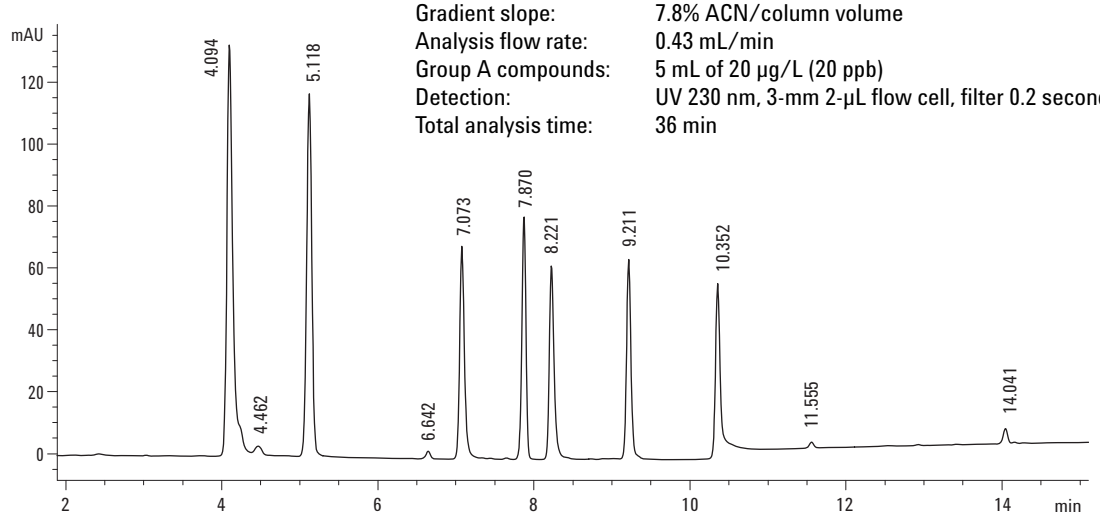


Figure 4. Trace enrichment (5 mL)of 20-ppb solution on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

The last peak in Figure 4 is missing due to a valve timing error that was not detected until sometime after the lab work was completed. Peak 8 was not eluted from the trace enrichment column before the valve switched offline for regeneration and equilibration. Note the baseline shift that occurs after peak 7, not seen in other autoSPE examples.

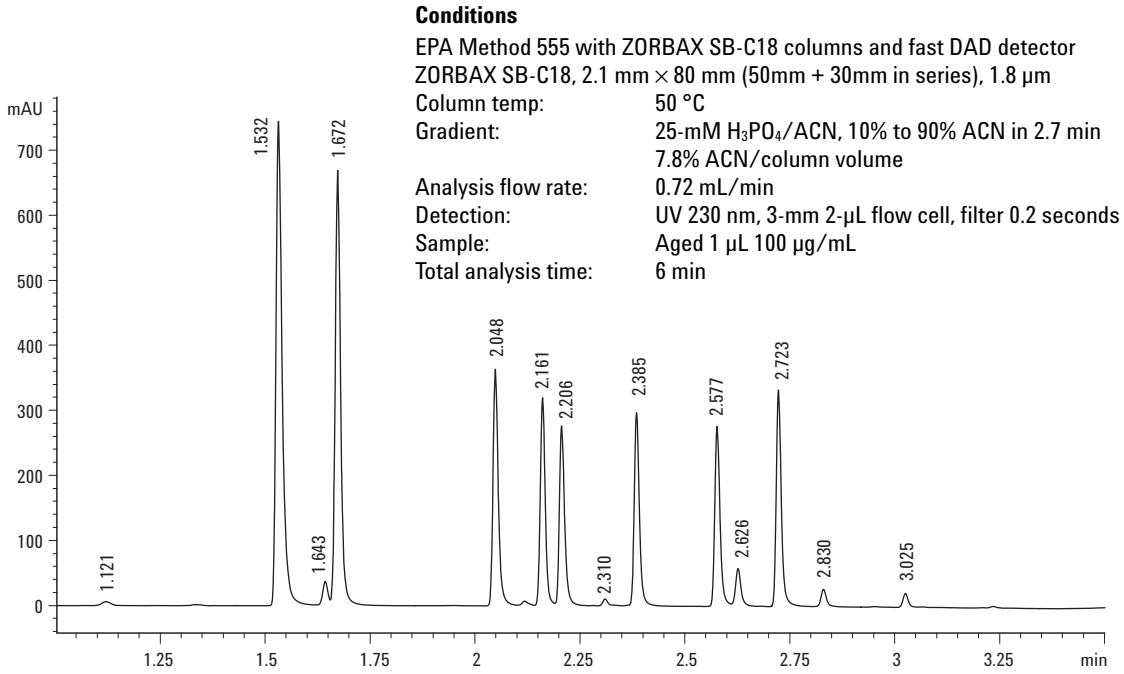


Figure 5. High-speed gradient separation of herbicides on 2.1 × 80 mm, 1.8-µm ZORBAX SB-C18.

In Figure 5 we see the combination of highest speed and resolution, using the full capability of the 1200 Rapid Resolution LC. Operating pressure was, at the maximum point, about 520 bar. We maintain comparable resolution to the original 4.6 × 250 mm, 5-µm method, a 60-minute run time, with an analysis time of only 6 minutes.

Conclusion

As is the case for many existing methods, it is both possible and practical to modernize this method to improve throughput and overall performance. Here we see the potential for a 10-fold increase in analysis speed and elimination of the loading pump scheme found in the original method. Approximately 1.3 mL of sample solution, injected to the autoSPE setup using the 2.1 × 80 mm configuration, is all that is needed to replace the 20-mL injection previously loaded through the pump. This approach can greatly improve productivity and ensure minimal sample cross-contamination.

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